

WHAT IS CLAIMED IS

- 5
1. Process of obtaining *in vitro* recombined polynucleotide sequences starting from a library of polynucleotide sequences characterized in that it includes the following steps:
- a) the fragmenting of a library of double-stranded polynucleotide sequences,
- b) the denaturation of the fragments possibly in the presence of one or several assembling templates,
- 10 c) the hybridization of said fragments with one or several assembling templates if it/they is/are not present in step (b),
- d) the ligation of said fragments in order to obtain recombined polynucleotide sequences,
- e) the selection of the recombined polynucleotide sequences having advantageous properties as compared to the corresponding properties of one or several reference sequences.
- 15
2. Process according to claim 1, characterized in that it includes at the end of step (d) and before step (e), the repeating of steps (b), (c) and (d) with the ligated and non-ligated fragments resulting from step (d).
- 20
3. Process according to one of claims 1 or 2, characterized in that it includes the separation of the recombined polynucleotide sequences from the assembling template or templates before step (e).
- 25
4. Process according to any one of the previous claims, characterized in that it includes the amplification of the double-stranded recombined polynucleotide sequences before step (e).
- 30

- 5
6. Process according to any one of the previous claims, characterized in that it includes before step (e), the cloning of recombined polynucleotide sequences possibly after separation of the recombined strands of the template or templates and obtaining the corresponding double strand.
- 10
7. Process according to any one of the previous claims, characterized in that the ends of the fragments generated at step (a) are such that there can be adjacent hybridization of these ends on the assembling template or templates at step (c) and ligation of these fragments with each other at step (d).
- 15
8. Process according to any one of the previous claims, characterized in that the polynucleotide sequences of the initial library have zones of homology either between themselves or with the assembling templates, so as to generate at step (a) ends of fragments which permit the adjacent hybridization of these ends on the assembling template or templates at step (c) and ligation of these fragments with each other at step (d).
- 20
9. Process according to any one of the previous claims, characterized in that the fragmenting of the polynucleotide sequences at step (a) is carried out in a controlled or a random manner.
- 25
10. Process according to any one of the previous claims, characterized in that step (a) consists of subjecting the polynucleotide sequences of the initial library to a hydrolysis by the action of one or several restriction enzymes.
- 30

11. Process according to any one of the previous claims,
characterized in that the degree of recombination and the position
of the points of recombination of the recombined polynucleotide
sequences are determined by the fragmentation described in step
(a) if it is carried out in a controlled manner.
12. Process according to any one of claims 1 to 9, characterized in
that the random fragmenting of the polynucleotide sequences at
step (a) is carried out by any known enzymatic or mechanical
means.
13. Process according to any one of the previous claims,
characterized in that enzymes capable of recognizing and of cutting
the non-hybridized ends of the fragments in a specific manner are
added at step (c) and/or step (d), when said ends overlap with other
hybridized fragments on the same template.
14. Process according to claim 13, characterized in that the enzyme
Flap endonuclease is added at step (c) and/or step (d).
15. Process according to any one of the previous claims, characterized
in that a ligase active at high temperature and preferably
thermostable is used at step (d).
16. Process according to claims 13 and 14, characterized in that the
endonucleases capable of recognizing and of cutting in a specific
manner the non-hybridized ends of the fragments added at step (c)
and/or at step (d) have the same thermoresistance and high
temperature activity properties as the ligase used at step (d).
17. Process according to any one of the previous claims characterized
in that the initial library of polynucleotide sequences is generated
starting from a native gene by steps of successive directed

mutagenesis, by error prone PCR, by random chemical
mutagenesis, by *in vivo* random mutagenesis, or by combining
genes of close or distinct families within the same or different
species in such a way as to arrange a variety of polynucleotide
sequences in an initial library.

5

18. Process according to any one of claims 1 to 16, characterized in
that the initial library of double-stranded polynucleotide sequences
is formed of synthetic sequences which will be fragmented at step
(a) or which can form the fragments of step (a).

10

19. Process according to any one of claims 1 to 11, 13 to 18,
characterized in that the step (a) consists of subjecting the initial
library to a hydrolysis by the action of restriction enzymes having a
large number of cutting sites on the polynucleotide sequences of
the initial library, or by combining several restriction enzymes.

15

20. Process according to any one of claims 1 to 9 and 12 to 18,
characterized in that the step (a) consists of a random treatment
with the DNase I of an initial library of partially heterologous,
double-stranded polynucleotide sequences.

20

21. Process according to any one of claims 12 or 20, characterized in
that fragments generated by a random treatment are used as
templates for each other, for the hybridization in the course of step
(c) or the RLR reaction of steps (c) and (d) simultaneously.

25

22. Process according to any one of claims 10 or 19, characterized in
that step (b) is carried out by combining at least two distinct libraries
of fragments generated separately at step (a) starting from the
same initial library by a treatment with different restriction enzymes.

30

23. Process according to claim 22, characterized in that the fragments obtained at step (a) by a treatment with restriction enzymes are used as templates for each other, for the hybridization during step (c) or of the RLR reaction of steps (c) and (d) simultaneously.

5

24. Process according to any one of claims 1 to 9, 11, 13 to 17 characterized in that the fragments of step (a) are obtained by amplification reactions directed by the polynucleotide sequence of the initial library.

10

25. Process according to claim 24, characterized in that the amplification reactions are carried out with oligonucleotide primers permitting generation of fragments whose ends are adjacent along the whole length of the assembling sequence.

15

26. Process according to claim 24, characterized in that the amplification reactions are carried out with oligonucleotide primers permitting generation of fragments having common sequences, said fragments serving as an assembling template for each other at step (b) or at step (c).

20

27. Process according to any one of the previous claims, characterized in that at step (a) the initial library is fragmented into n fragments, n being greater than or equal to 3.

25

28. Process according to any one of claims 1 to 18, 24 to 27, characterized in that the assembling template at step (b) or (c) is a polynucleotide sequence resulting from the initial library or a single or double-stranded consensus sequence of said library.

30

29. Process according to any one of claims 1 to 19, 24 to 27, characterized in that single or double-stranded oligonucleotides complementary just at the 3' end of one fragment and at the 5' end

of the adjacent fragment are used as assembling template at steps
(b) or (c).

5 31. Process according to any one of the previous claims, characterized
in that single or double-stranded oligonucleotides, of variable
length, are added at step (b) or (c) in addition to the template.

10 32. Process according to any one of the previous claims,
characterized in that, before step (e) the recombined polynucleotide
sequences are separated from the assembling template thanks to a
label present on the assembling template or on the recombined
polynucleotide sequences.

15 33. Process according to any one of the previous claims,
characterized in that the recombined polynucleotide sequences
obtained at step (d) and possibly cloned are screened by any
appropriate means in order to select the recombined polynucleotide
sequences or the clones having advantageous properties as
compared to the corresponding properties of the reference
20 sequences.

25 34. Process according to any one of the previous claims,
characterized in that the initial library of polynucleotide sequences
is formed by one or several limited librarys prepared by a process
according to any one of claims 1 to 33, possibly mixed with other
polynucleotide sequences.

30 35. A recombined polynucleotide sequence having one or several
advantageous properties as compared to the corresponding
properties of reference sequences obtained and selected by a
process according to anyone of claims 1 to 34.

36. A vector containing a polynucleotide sequence according to claim 35.

5 37. A cellular host transformed by a recombined polynucleotide sequence according to claim 35 or by a vector according to claim 36.

10 38. A protein encoded by a recombined polynucleotide sequence according to claim 37.

39. A library formed from recombined polynucleotide sequences according to claim 35, or of the vector according to claim 36, or of host cells according to claim 37, or of proteins according to claim 38.